

POTENT AND SELECTIVE HUMAN NEURONAL NITRIC OXIDE SYNTHASE INHIBITORS

CROSS-REFERENCED TO RELATED PATENT APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/980,735, filed Feb. 24, 2020 and to International Application No. PCT/US2020/019466, filed Feb. 24, 2020, the contents of which are incorporated herein by reference in their entireties.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under grant GM049725 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] The field of the invention relates to 2-aminopyridine derivative compounds for use as inhibitors of nitric oxide synthase. In particular, the field of the invention relates to 2-aminopyridine derivative compounds for use as inhibitors of nitric oxide synthase, which are formulated as pharmaceutical compositions for treatment of neurological diseases or disorders, which may include but are not limited to Alzheimer's, Parkinson's, and Huntington's diseases, amyotrophic lateral sclerosis, cerebral palsy, stroke/ischemic brain damage, and migraine headaches.

[0004] In some aspects, the disclosed subject matter relates to methods for treating neurological diseases and disorders and particularly neurodegenerative diseases. Neurodegenerative diseases, such as the commonly known Alzheimer's, Parkinson's, and Huntington's diseases, are characterized by a gradual degeneration and death of neurons in central nervous system (CNS), causing problems in muscular movements and mental functioning of patients. Despite acute medical needs, comprehensive treatments for these diseases are still very limited.^{1, 2} One of the most difficult challenges in CNS drug development is to effectively deliver therapeutic drugs into the human brain, mainly because of the presence of a blood brain barrier (BBB) located at the interface of blood vessels and brain tissues.³ The BBB is composed of a layer of endothelial cells with tight junctions that prevents the access of external toxins, and therefore protects the brain and preserves its optimal physiological environment. This cell layer, however, also limits the access of valuable therapeutic drugs into the brain.⁴ The major pathway for CNS drugs to cross the BBB is a passive diffusion through its lipid membrane. In addition to the tight junctions of endothelial cells, high expression levels of efflux transporters on the BBB, especially P-glycoprotein (P-gp), contributes greatly to the limited brain exposure of CNS drugs.⁵ Consequently, it is necessary in CNS drug development to establish a strategy that includes a combination of increasing the passive permeability and lowering the P-gp mediated efflux.^{3, 6, 7}

[0005] Neuronal nitric oxide synthase (nNOS) has been validated as a promising therapeutic target in the development of new treatments for neurodegenerative diseases.⁸⁻¹⁰ In brain, nitric oxide (NO) produced by nNOS participates

in neuronal transmissions.¹¹ The overproduction of NO in cells, however, is harmful. Particularly, excess NO formed by overactivated nNOS in CNS can cause excessive nitration and nitrosylation of proteins, leading to their misfolding and aggregation.¹² Additionally, the reaction of NO with superoxide anion creates a strongly oxidizing reagent, peroxynitrite, which damages DNA and causes lipid peroxidation. These processes lead to the nerve cell death and the impairment in neuronal transmissions.^{13, 14} Limiting NO production through inhibiting nNOS, therefore, could become an essential approach to protect neurons and potentially cure certain neurodegenerative diseases.^{15, 16}

[0006] nNOS is a homodimeric enzyme with each monomer containing one C-terminal reductase domain and one N-terminal oxygenase domain. The C-terminal reductase domain includes nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN), whereas the N-terminal oxygenase domain includes a non-catalytic zinc, tetra-hydrobiopterin (H₄B), and a heme. These two domains are connected to each other by a calmodulin domain. When dimerization occurs, an electron flow is facilitated from the reductase domain to the oxygenase domain, through which L-Arg gets oxidized to L-Cit and NO is released.^{15, 17} Facilitating a molecule to compete with L-Arg binding at the active site of the enzyme is one of the fundamental approaches to inhibit nNOS.¹⁶ The challenges of this task involve not only the potency of nNOS inhibitors, but also relate to their binding selectivity for nNOS over both eNOS and iNOS, the two isoforms that share very similar structural features to that of nNOS.^{18, 19} It is necessary to avoid over-inhibition of both eNOS and iNOS because eNOS inhibition can result in cardiovascular failure while iNOS inhibition can cause a disruption in the immune system.²⁰

[0007] In recent years, the inventors' efforts in achieving nNOS inhibitors with excellent potency and high isoform activity have led to a promising class of molecules bearing a 2-aminopyridine scaffold. Using this molecular scaffold, the inventors obtained nNOS inhibitors that exhibit excellent activity at concentrations in the <30 nM range.^{15, 21, 22} The first generation of nNOS inhibitors bearing a 2-aminopyridine scaffold, however, showed poor predicted permeation through the BBB as revealed by very little Caco-2 permeability.²³ Recently, the inventors were able to improve the cell membrane permeability of 2-aminopyridine nNOS inhibitors, while retaining their high inhibitory activity for nNOS. The inventors previously identified a lead compound (1, FIG. 1), which shows an excellent potency and selectivity to human nNOS (K_i $_{hNOS}$ =30 nM; $hNOS/hNOS$ =2799) and displays an efflux ratio (ER) of 5.9 in Caco-2 assay.²⁴ In order to move forward in CNS drug development, the cell membrane permeability of 2-aminopyridine nNOS inhibitors must be further improved with a required ER of <2.5 for being a likely CNS(+) drug.^{7, 25}

SUMMARY

[0008] Disclosed are 2-aminopyridine compounds, pharmaceutical compositions and methods of treating diseases or disorders associated with nitric oxide synthase (NOS) activity. Diseases and disorders treated by the disclosed compounds, pharmaceutical compositions, and methods may include neurological diseases or disorders. Neurological diseases or disorders treated by the disclosed 2-aminopyridine compounds may include, but are not limited to neuro-